Doctoral Student Research Committee (DSRC)

4th meeting

Period: 12th June, 2023 to 25th April 2024

Ph.D. Research Scholar: Ruchi Singh

Registration no: 22//Ph.D./DPSRU/2020

Date of Registration: 01/01/2021

Formulation and Evaluation of Novel Nano Nutricosmetics for skin and hair

Supervisor: Prof. Harvinder Popli

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SCHOOL OF PHARMACEUTICAL SCIENCES

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Executive Summary (4th DSRC)

Name of Research Scholar: Ruchi Singh **Name of Supervisor:** Prof Harvinder Popli

Registration No.: 22/PhD/DPSRU/2020 **Date of DSRC:** 26th April 2024

Title of thesis: Formulation and Evaluation of Nano Nutricosmetics for Skin and hair

Abstract of the thesis (500-800 words, in structured manner with separate paragraphs for Introduction ending with Objectives; Methodology; Results; Conclusions and Outcomes of the overall work done): Introduction :

- Pollution has a great impact on skin cells. The negative impact can be observed at the skin surface, the stratum corneum, which is normally colonized with residual microorganisms. In the presence of pollutants, the skin microbiome changes for the benefit of pathogenic bacteria. Moreover, environmental contamination enhances the production of reactive oxygen species (ROS), which depletes the content of antioxidants in the skin. This causes disturbance in disturbance in the redox balance, causing stress to the cells. Some pollutants also tend to permeate through the stratum through the stratum corneum into deeper skin layers. There, they act as a ligand for the Aryl hydrocarbon receptor (AhR), which takes part in mediating toxic effects of pollutants. The alterations of microflora, oxidative stress and the activation of AhR cause the induction of an inflammatory cascade in the skin. The increased production of pro-inflammatory cytokines, such as interleukin1ß or interleukin 8, greatly impacts the interleukin1ß or interleukin 8, greatly impacts the biological function of the cells, resulting in deterioration of skin appearance.

Objectives :

With an aim to combat the effects of urban pollution on skin and hair , novel Nano Nutricosmetic formulations were developed.

Objective 1: Development and optimization of B12/FA Loaded Transferosomes loaded Antipollution skin care

Objective 2: Development and optimization of Rosemary Oil and Pea Protein Nano Emulsion for Hair care Application

Methodology :

Objective 1: Development and optimization of B12/FA Loaded Transferosomes loaded Antipollution skin care

The APIs were analyzed using the developed and validated UV and HPLC method (ICHQ2R1) , through Preformulation studies were carried out including , FTIR analysis, solubility determination, partition coef. QTPP was defined and CQA was established. The Initial risk assessment was carried out using ishikawa fishbone diagram and severity , uncertainty analysis. 3^2 full factorial design was used for optimization of the formulations. The optimized transfersomes were evaluated for particle size using TEM and DLS, PDI, Zeta potential, Lipid vesicle flexibility, entrapment efficiency. The optimized Transferosomes were incorporated into a moisturizing cream formulation. For claim substantiation of Antipollution effect ,

DPPH radical scavenging , Anticollagenase, and Antielastase activity was carried out. HET CAM assay was performed. Stability indicating analytical method was developed for evaluation of B12 and FA during shelf life determination studies. Acid Degradation, Base degradation, Peroxide Degradation, Thermal Degradation was carried out. Stability studies was carried out as per ICH guideline at 30±2°C/65%RH, 40± 2°C/75±5%RH, 2-8°C (Refrigerator). % Assay, Visual Appearance, pH, Leakage, Texture, Phase separation were recorded as stability parameters. The Developed Cream was evaluated as per BIS standards IS 6608.

Objective 2: Development and optimization of Rosemary Oil and Pea Protein Nano Emulsion for Hair care Application

In order to develop a multifunctional hair care formulation Pea protein loaded Rosemary oil nano emulsion was developed and optimized using D-optimal design, Globule size and percent transmittance were studied as the dependent variables , Concentration of oil , surfactant and cosurfactant were studied as independent variables. DHT inhibition studies were performed using Ex-vivo extraction of Testosterone from Goat prostrate and compared with Finasteride. The developed Rosemary Oil and Pea Protein Nano Emulsion was incorporated into hair serum and hair mask formulation for ease of application. Sensory mapping studies were carried out to evaluate the consumer perception about Spreadibility, softness, ease of application and after feel appeal.*Invivo* hair growth potential in testosterone induced Androgenetic Alopecia was carried out in Male Wistar rats. 3 groups of 3 animals each were used in the study. Androgenetic alopecia was induced by subcutaneous injection of testosterone (10mg/kg) for 21 days. Each rat of group II and III were anesthetized by Ketamine i.p. Injection then hair of back of rat was removed by help of hair removal cream to induce diffusive alopecia. Treatment in group II, III was started after development of alopecia. Significant hair loss seen in mice treated with Testosterone. Post 56 days, the mice were sacrificed and the histopathology studies was carried out. % of hair follicle in anagen and telogen phase was evaluated. Stability studies were carried out as per ICH guideline . Hair serum , Hair mask were evaluated as per BIS standards IS 7123 and IS 7679.

Results :

Objective 1: Development and optimization of B12/FA Loaded Transferosomes loaded Antipollution skin care

The optimized transferosomes were found to have particle size in the range of 120-200 nm and Zeta potential in the range of 45-51mv. The PDI was found to be below 0.3. No change in size and volume was observed after extrusion through 0.1micron filter confirming the flexibility of the Transferosomes. TEM confirmed the particle size (120-190nm) shown by DLS evaluation.

The combination of B12 and Folic Acid Transferosomes exhibited more than 85% DPPH inhibition , 72% collagenase inhibition and 68% elastase inhibition at the 2 mg/ml solution, thereby confirming the synergistic antipollution , antiaging and antioxidant activity of B12 and Folic acid Transferosomes.

No irritation on potential was observed using the HET CAM assay.

B12 transfersomes exhibited 48% higher flux and Folic acid Transfersomes exhibited 69% higher flux as compared to plain API

Both B12 and FA were stable in Base, Acid and Thermal conditions. Both B12 and FA exhibited shift in peak and lambda max in peroxide due to their radical scavenging activity, however no loss of potency was observed. The developed product was stable for 18 months period . The cream successfully passed the test for thermal stability, meeting the specified requirements. The pH of the cosmetic cream fell within the acceptable range of 4.0 to 9.0, with a measured value of 6.4 ±0.5, indicating compliance with the standard. The cosmetic cream

exceeded the minimum requirement of 5.0% for total fatty substance content, with a measured value of 24.3%, indicating a rich formulation. The total residue content of the cosmetic cream was within the specified limit of 10%, with a measured value of 32.4%. The cosmetic cream passed the test for heavy metals, with the color of the test solution being less than the standard, indicating absence of lead contamination. The arsenic content in the cosmetic cream was well below the maximum allowable limit of 2 ppm, with a measured value of less than 0.02 ppm, indicating absence of arsenic contamination. The cosmetic cream exhibited a total viable count of 62.4 CFU/g, which was within the allowable limit of 1000 cfu/g. The presence of gram-negative pathogens in the cosmetic cream was below the specified limit of less than 10 CFU/g, with a measured value of 37.8 CFU/g.

Objective 2: Development and optimization of Rosemary Oil and Pea Protein Nano Emulsion for Hair care Application

UV method for pea protein was developed and validated by ICH Q2R1. The developed Rosemary Oil and Pea Protein Nano Emulsion was incorporated into hair serum formulation for ease of application. Sensory mapping studies were carried out to evaluate the consumer perception about Spreadibility, softness , ease of application and after feel appeal.

In mice treated with oral finasteride 32% follicles were in telogen phase and 68% in Anagen as compared to 11% in telogen phase and 89% in anagen phase in the group treated with the developed formulation.

The hair mask successfully passed the test for thermal stability, meeting the requirement to withstand specified thermal conditions. The pH of the hair mask fell within the acceptable range of 5.0 to 9.0, with a measured value of 5.5 ±0.5, indicating compliance with the standard. The hair mask exceeded the minimum requirement of 15.0% for total fatty substance content, with a measured value of 34.85%. The water content of the hair mask was within the specified limit, with a value of 58.46%, meeting the maximum allowable limit of 85%. It passed the test for rancidity, as evidenced by the absence of a pink color in the solution, indicating the absence of rancid odors or flavors. The mask exhibited a low microbial count, with a total viable count of 18 CFU/g, well below the maximum allowable limit of 1000 cfu/g, indicating good microbial quality. The hair mask successfully met the requirements outlined by BIS guidelines IS 7123 demonstrating its quality, stability, and safety for consumer use.

The acid value of the hair serum was well below the acceptance criterion of less than 1, with a value obtained of 0.1, indicating low levels of free fatty acids. The serum exhibited a low microbial count, with only 23 microorganisms per gram detected, significantly lower than the maximum allowable limit of less than 1000 microorganisms per gram, indicating good microbial quality and hygiene. The peroxide value was substantially below the maximum allowable limit of 10 milliequivalents/1000 g, with a value obtained of 0.2 milliequivalents/1000 g, suggesting minimal oxidative deterioration. The hair serum passed the test for heavy metal analysis. Lead (Pb) levels were well below the standard, with a value obtained of less than 0.02 ppm, and arsenic (As2O3) levels did not exceed the maximum limit of 20 ppm, indicating the absence of harmful heavy metal contamination.

Conclusion :

In conclusion, the project successfully developed advanced skincare and haircare solutions tailored to combat the detrimental effects of urban pollution and androgenetic alopecia.. These novel nano nutricosmetic

formulations not only offer effective protection against environmental stressors but also promote healthy skin and hair, meeting the evolving needs of modern consumers.

Recommendations of 3rd DSRC	Compliance of 3rd DSRC (write clearly what changes were done)
1. The *Invivo* and Stability Data presented by the candidate was appreciated by the experts 2. One of the experts suggested to carry out microbial evaluation of the developed products 3. Experts also suggested to file patent for the developed product before publications	Microbial Evaluation was completed and presented in 4th DSRC as per BIS guidelines Patent filing is in process and will be completed before thesis submission

Recommendations of URAC after 3rd DSRC	Compliance
3rd URAC Pending	

Recommendations of 4th DSRC:

The candidate presented nutricosmetic for skin and hair based on market analysis the targeted formulation was intended for urban pollution. In the period of 4th DSRC, the candidate presented stability data as per ICH guidelines and evaluation as per BIS standards. The formulated product followed all the guidelines of QbD, ICH, BIS and the final product is an advanced anti-pollution all in one skin protection against pollution, smoke, smog, UVA and UVB radiation. The product will have commercial applicability for extrinsic aging, pigmentation and tanning.

The second product was a nutricosmetic for hair, and the targeted indication was androgenetic alopecia. The animal study and the choice of ingredients indicates the potential as given in the form of serum and mask. Both the products can be scaled for commercial use in future by industry. The studies are completed and can be submitted for doctoral thesis. It is suggested to validate with molecular docking. Publication is accepted and other publication is communicated. Good presentation of the remaining scientific work. Sensory and Textural analysis was done and found satisfactory. Submission of patent application is advised before submission of the final thesis.

Plagiarism report: 6% similarity (Report attached)

List of Publications (Title pages to be attached as annexure):

1. Development and optimization of Clitoria Teratea synthesized silver nanoparticles and its application to nanogel systems for Wound Healing. R Jain, R Singh, R Badhwar, T Gupta, H Popli Drug Development and Industrial Pharmacy, 1-35
2. Implementation of quality by design (QbD) approach in development of qct-smedds with combination of AgNPs for diabetic foot ulcer management R Badhwar, R Singh, H Popli Indian J. Pharm. Educ. Res 55, 1207-1223

Publications communicated:

1. A QbD methodology for the design and optimization of Peppermint Oil Loaded SMEDDS for the treatment of Androgenetic Alopecia" (Submission ID: DDTR-D-24-00372)
2. Design Development and Optimization of B12 and Folate Loaded Transferosomes and evaluation of its anti- pollution activity: A QbD based Approach. (Submission ID: DDIP-CLT 2024))

List of conferences attended (Proofs to be attached as annexure):

1. Participated in 3 Day " International Symposium on Nanoscience and Nanotechnology, Organized by Amity center of Nanotechnology, Amity university, Haryana 7th-9th June 2020
2. Participated in AICTE/ISTE sponsored, One-week induction program on Multidisciplinary and translational drug development QbD in Pharma Development, organized by Anjuman Islam's Kalsekar Technical Campus , School, of Pharmacy , New Panvel , 24th-29th May 2021
3. Presented Research Paper at Pharma Anveshan 2023, National pharmacy Education Day, Organized by Pharmacy Council of India, Vigyan Bhavan 6th March 2023
4. Presented research paper at Global Pharma Healthcare Technology Summit 2023, 15-17th February, 2023, Hyatt Centric New Delhi

Patents Published / granted (Proofs to be attached as annexure):

Signature of Student

Signature of Supervisor

Delhi Pharmaceutical Sciences And Research University, New Delhi-17 , India

Department of Pharmaceutics

Report of The DSRC

Name of the Research Scholar : Ruchi Singh

Registration Number : 22/Ph.D/DPSRU/2022.

Conduct of Research Scholar : – Good –

Overall assessment of the progress and specific comments :

The candidate presented nutricosmetics for skin and hair based on market analysis, the targetted formulation was intended for urban pollution. In the period of 4th DSRC The candidate presented stability data as per ICH guidelines and evaluations as per BIS standards. The formulated product followed all the guidelines of QbD, ICH, BIS and the final product is an advanced antipollution all in one skin protection against pollution, smoke, smog, UVA and UVB radiation. The product will have commercial applicability for extrinsic aging, pigmentation and tanning.

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Expert 1	Expert 2	Supervisor
Prof. Sanju Nanda	**Dr Simrata Bedi**	**Prof (Dr). Harvinder Popli**
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Date: Nanda 26.4.24. (Sanju Nanda)		26/4/24

Delhi Pharmaceutical Sciences And Research University, New Delhi 17, India

Department of Pharmaceutics

Report of The DSRC

Name of the Research Scholar : Ruchi Singh

Registration Number :

Conduct of Research Scholar : - Good -

Overall assessment of the progress and specific comments :

The candidate presented outcome of skin and hair based on market analysis, the targeted formulation was intended for urban pollution. In the presentation the candidate presented stability data as per ICH guidelines and evaluations as per BIS standards. The formulated product followed all the guidelines of QbD, ICH, BIS and the final product is an advanced anti-pollution all in one skin protection against pollution, smoke, smog, UVA and UVB radiation. The product will have commercial applicability for extrinsic aging, pigmentation and tanning.

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Nanda 26.4.24. (Sanju Nanda)		26/4/24.